

24 TOPIRAMATE AND QUETIAPINE QUANTITATION AND CONFIRMATION BY LCMS	Page 1 of 4
Division of Forensic Science TOXICOLOGY TECHNICAL PROCEDURES MANUAL	Amendment Designator:
	Effective Date: 31-March-2004
<p style="text-align: center;">24 TOPIRAMATE AND QUETIAPINE QUANTITATION AND CONFIRMATION BY LCMS</p> <p>24.1 Summary</p> <p>24.1.1 Topiramate and quetiapine are extracted from biological samples by making the samples basic with saturated borate buffer and extracting with toluene/hexane/isoamyl alcohol (THIA). An aliquot of the extract is analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).</p> <p>24.2 Specimen Requirements</p> <p>24.2.1 2 mL blood, fluid or tissue homogenate.</p> <p>24.3 Reagents and Standards</p> <p>24.3.1 Quetiapine, 1 mg/mL</p> <p>24.3.2 Topiramate, 1 mg/mL</p> <p>24.3.3 Mepivacaine, 1 mg/mL</p> <p>24.3.4 Sodium tetraborate decahydrate</p> <p>24.3.5 Hexane</p> <p>24.3.6 Isoamyl alcohol</p> <p>24.3.7 Methanol</p> <p>24.3.8 Toluene</p> <p>24.3.9 Ammonium acetate</p> <p>24.4 Solutions, Internal Standard, Calibrators and Controls</p> <p>24.4.1 10 mM Ammonium Acetate: Weigh 0.389 g ammonium acetate. Transfer to 500 mL volumetric flask and QS to volume with dH₂O</p> <p>24.4.2 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH₂O until no more dissolves after shaking vigorously.</p> <p>24.4.3 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol.</p> <p>24.4.4 Drug stock solutions:</p> <p>24.4.4.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and QS to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.</p> <p>24.4.5 Working standard solution for topiramate (0.1 mg/mL): Pipet 1 mL of the 1 mg/mL stock solution of topiramate into a 10 mL volumetric flask and QS to volume with methanol.</p>	

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24.4.6 Working standard solution for quetiapine (0.01 mg/mL): Pipet 100 µL of the 1 mg/mL stock solution of quetiapine into a 10 mL volumetric flask and QS to volume with methanol.

24.4.7 Working internal standard solution (0.1 mg/mL mepivicaïne): Pipet 1 mL of the 1 mg/mL stock solution of mepivicaïne into a 10 mL volumetric flask and QS to volume with dH₂O.

24.4.8 To prepare the calibration curve, pipet the following volumes of the 0.1 mg/mL topiramate working solution and 0.01 mg/mL quetiapine working solution into appropriately labeled 16 x 125 mm screw cap test tubes. Evaporate standards to dryness under nitrogen. Add 2 mL with blank blood to obtain the final concentrations listed below.

Amount of 0.1 mg/mL topiramate standard (µL)	Final concentration of topiramate (mg/L)	Amount of 0.01 mg/mL quetiapine standard (µL)	Final concentration of quetiapine (mg/L)
1000	50	1000	5
400	20	400	2
200	10	200	1
100	5	100	0.5
40	2	40	0.2
20	1	20	0.1

24.4.9 Controls

10.1.1.1 Topiramate and Quetiapine Controls. Control may be from a external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

24.4.9.1 Negative control. Blood bank blood or equivalent determined not to contain topiramate, quetiapine or mepivicaïne.

24.5 Apparatus

24.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

24.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

24.5.3 Centrifuge capable of 2000-3000 rpm

24.5.4 Nitrogen evaporator with heating block

24.5.5 Vortex mixer

24.5.6 GC autosampler vials with inserts

24.5.7 LC/MS: Agilent Model 1100 LC-MSD

24.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

24.5.7.1.1 Elution Conditions

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- 24.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 μ M particle size
- 24.5.7.1.1.2 Column thermostat: 35° C
- 24.5.7.1.1.3 Solvent A: 55% 10 mM ammonium acetate
- 24.5.7.1.1.4 Solvent B: 45% methanol
- 24.5.7.1.1.5 Isocratic elution, stop time: 13.00 min

Time (min)	Solv. B	Flow
0.00	45	0.45
4.00	80	0.45
8.00	80	0.45
9.00	45	0.45

24.5.7.1.2 Spray Chamber

- 24.5.7.1.2.1 Ionization Mode: Electrospray
- 24.5.7.1.2.2 Gas Temperature: 350° C
- 24.5.7.1.2.3 Drying Gas (N₂): 12.0 L/min
- 24.5.7.1.2.4 Nebulizer pressure: 30 psig
- 24.5.7.1.2.5 Vcap (Positive): 4000 V

24.5.7.1.3 Selected Ion Monitoring (quantitation ions)

- 24.5.7.1.3.1 Polarity: Positive
- 24.5.7.1.3.2 Injection volume: 2 μ L

Time (min)	Group Name	SIM Ion	Fragmentor	Gain EMV	SIM Resol.	Actual Dwell
0.00	topiramate	264	150	1.5	Low	218
		282	150		218	
		340	150		218	
		<u>357</u>	150		218	
4.30	mepivacaine	98	150	0.5	Low	439
		<u>247</u>	150		439	
6.00	quetiapine	210	250	0.5	Low	218
		253	250		218	
		279	250		218	
		<u>384</u>	250		218	

24.6 Procedure

- 24.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 24.6.2 Prepare calibrators and controls.
- 24.6.3 Add 2 mL case specimens to the appropriately labeled tubes.
- 24.6.4 Add 40 μ L 0.1 mg/mL mepivacaine internal standard working solution to each tube for a final concentration of 2mg/L.

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<p>24.6.5 Add 2 mL saturated borate buffer and 6 mL extract solvent (78:20:2 THIA) to each tube.</p> <p>24.6.6 Cap and rotate tubes for 30 minutes.</p> <p>24.6.7 Centrifuge at approx 2500 rpm for 15 minutes. Transfer organic upper layer (THIA) to appropriately labeled conical bottom test tubes.</p> <p>24.6.8 Evaporate samples to dryness at approximately 50° C under nitrogen.</p> <p>24.6.9 Reconstitute samples in 100 µL methanol. Vortex briefly. Transfer to GC autosampler vials for analysis by LCMS.</p> <p>24.7 Calculation</p> <p>24.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.</p> <p>24.8 Quality Control and Reporting</p> <p>24.8.1 See Toxicology Quality Guidelines</p> <p>24.9 REFERENCES</p> <p>24.9.1 M Contin, R Riva, F Albani and A Baruzzi. Simple and rapid liquid chromatographic-turbo ion spray mass spectrometric determination of topiramate in human plasma. J Chrom B 761: 133-137, 2001.</p> <p>24.9.2 J Pearson and R Steiner, in-house development.</p>	